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INFLUENCE OF GROWTH REGULATORS ON PESTICIDE UPTAKE

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This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

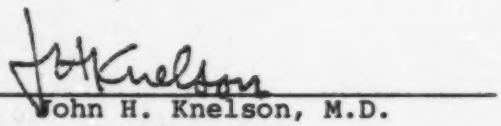
FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The acceptance of an integrated pest management approach for the control of undesirable species in the agricultural environment is a major advance in our attempt to increase agricultural productivity while decreasing untoward effect on our environment. To this end increasing the effectiveness of our pest control agents can reduce the hazards associated with environmental contamination.

This study was conducted to evaluate the use of plant hormones in reducing the amount of herbicide necessary to control certain noxious weeks.



John H. Knelson, M.D.
Director,
Health Effects Research Laboratory

ABSTRACT

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CONTENTS

Section		Page
I.	Conclusions	1
II.	Recommendations	3
III.	Introduction	3
IV.	Methods and Materials	6
V.	Results and Discussion	8
VI.	References	17
VII.	Publications	20

FIGURES

1. Effect of GA on the uptake of naptalam by bean plants.
2. Effect of GA on the uptake of naptalam by different parts of the bean plant.
3. Effect of 2,4-D and PCPA on the uptake of naptalam by the bean plant.
4. Effect of 2,4-D on the uptake of naptalam by different parts of the bean plant.
5. Effect of PCPA on the uptake of naptalam by different parts of the bean plant.

TABLES

1. Amount of radioactivity extracted from cranberry plants treated with naptalam-C-14 and chlorpropham-C-14.

ACKNOWLEDGMENTS

The support of the project by the Public Health Service and by the Environmental Protection Agency is acknowledged with sincere thanks.

SECTION I

CONCLUSIONS

The discovery that plant growth regulators, both natural and artificial, are capable of accelerating the uptake of a herbicide by bean plants may have some practical significance. If we assume that other herbicides and plants are so influenced then more efficient herbicidal activity can be expected when both herbicide and growth regulators are applied together. Also, and perhaps more important, the amounts of different herbicides necessary to control certain noxious weeds could be reduced.

The spectrum of weeds controlled by certain herbicides could also be broadened to a significant degree by their simultaneous application with growth regulators. Finally, it is reasonable to assume that the uptake of systemic insecticides could be accelerated by plant growth regulators. Chewing and sucking pests could then be controlled in a much more efficient manner.

SECTION II

RECOMMENDATIONS

1. The feasibility of using growth regulators to enhance the toxic efficiency of pesticides in commercial agriculture should be given serious examination.

2. Where growth regulators are now used in agriculture, their effect on the uptake of other compounds in the soil--pesticides, mineral elements, water, etc.--should be examined.

SECTION III

INTRODUCTION

The availability of soil-applied pesticides is under the control of several factors, the most important of which are volatilization, biological and chemical decomposition, and adsorption to soil particles. These factors make available to the target plant only a small amount of the pesticide that is actually delivered to the soil. The available pesticide must be absorbed then by the target plant and translocated to a site(s) of activity. Presumably, the amount of pesticide absorbed and its distribution in the plant determine its toxic efficiency.

A number of papers have been published in recent years describing the influence of plant growth hormones on the uptake, translocation, and accumulation of materials commonly found in plants. Mothes and Engelbrecht (10,11,12) demonstrated that kinetin-treated areas of tobacco leaves possess the ability to attract amino acids and other metabolites from untreated areas of the leaves. This they referred to as "kinetin-induced directed transport." The study with tobacco leaves was supported by Gunning and Barkley (3) who showed that kinetin is capable of directing, in a similar manner, the movement of amino acids in barley leaves. Promotion by kinetin of the uptake of K^+ and Rb^+ into detached sunflower cotyledons and the accelerated transport of ^{22}Na in the direction of kinetin-treated parts of corn leaves

have been demonstrated (5,6,7,13). In addition, Kriechmann (9) has recently shown that the application of kinetin to a young orange fruit (cv. Washington Navel) enhances its ability to import photosynthetic assimilates.

The plant growth regulator indole-3-acetic acid (IAA) has also been found to be quite active in accelerating the transport of materials in the plant. For example, application of IAA to the tips of decapitated pea and bean plants considerably accelerated the uptake and accumulation of ^{32}P-phosphate and ^{14}C-sucrose in the decapitated internodes (1,2). In a similar pair of studies, Seth and Wareing (15,16) demonstrated that the stimulatory influence of auxin in this respect could be greatly enhanced by applying gibberellic acid (GA) and kinetin simultaneously with IAA to the decapitated internode. Work by Osborne and Hallaway (14) showed that the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) can direct the translocation of organic materials to areas where it is applied on detached leaves of Prunus.

Several investigators have studied the influence of GA on the uptake and translocation of a number of compounds. If immature clusters of 'Black Corinth' grapes are dipped in GA or 4-chlorophenoxyacetic acid (PCPA)--a synthetic auxin--there is a significant increase in the movement of photosynthetic products into the young fruits (17,19). In another study, the

application of GA to the tips of decapitated soybean plants had a marked stimulatory effect on the translocation of ^{14}C-sucrose out of leaves previously exposed to $^{14}CO_2$ (4). Finally, Kannan and Mathew (8) found that when bean roots were pretreated with GA, the absorption of Fe applied to the primary leaf and subsequent transport to the trifoliate leaves were increased.

SECTION IV

METHODS AND MATERIALS

Bean plants are planted in clay pots containing a nine-to-one mixture of sand and peat and then germinated and grown in a Percival growth chamber (Model F540) under a photoperiod of 16 hr light (800 ft.c.) and 8 hr dark. The temperature during the light period is maintained at 27°C and during the dark period at 21°C. Eleven to fourteen-day-old bean plants are matched into pairs with four pairs of plants constituting an experiment. One plant from each pair represents a control.

The selected plants are placed into wide-mouth, 250 ml erlenmeyer flasks that hold 150 ml of aerated nutrient solution (Hoagland No. 1) and a known amount of pesticide. The growth regulator to be tested is then added to one set of flasks (either to the foliage or directly to the nutrient solution). Each flask will contain two plants held in place with a cotton plug. The flasks then are placed in a growth chamber under continuous light (800 ft.c.) and a temperature of 24°C for 24 hr. After this period the plants are divided into the following sections: leaves (aerial portion from and including the first true leaves up) lower epicotyl (area of the stem between the cotyledonary node and the first true leaves) hypocotyl, and the root system. The plant tissues are cut into small pieces and dried at 45°C for 24 hr in preparation for extraction of the pesticide. Before

being extracted the dried plant tissues are ground into fine powder (20 mesh screen) by a Wiley Mill. Each experiment is replicated at least three times.

Naptalam content of the plant tissue is determined with only slight modification by the method of Smith and Stone (18). The method relies on the basic hydrolysis of naptalam in boiling 30% sodium hydroxide and the steam distillation of 1-naphthylamine. Under the conditions of our study, steam distillation of the amine is complete when 15 ml of distillate have been collected.

In preparation for color development, 5 ml of glacial acetic acid and 5 ml of distilled water are added to the 15 ml of distillate. Ten drops of freshly prepared diazonium reagent then are added to the diluted distillate. The diazonium reagent is composed of equal volumes of 1% sulfanilic acid and 0.12% sodium nitrite that have been allowed to react for about 4 min. The amine present in the distillate couples with diazotized sulfanilic acid to give an azo dye. After 30 min the intensity of the red color formed is read at 534 nm with a Beckman DU spectrophotometer. A linear relationship exists between the intensity of color and the amount of 1-naphthylamine present.

SECTION V

RESULTS AND DISCUSSION

The stimulatory influence of plant growth hormones on the uptake and accumulation of different compounds has been demonstrated in a number of relatively recent studies. However, in all of these studies the transport material has always been something that is found as a natural compound. In our studies we have shown that GA, 2,4-dichlorophenoxyacetic acid (2,4-D), and 4-chlorophenoxyacetic acid (PCPA) can dramatically accelerate the uptake and accumulation of the herbicide naptalam.

As shown in Figure 1 all concentration of GA from 2.9×10^{-6} to 8.6×10^{-4}M effectively stimulated naptalam uptake. Pretreatment of bean plants with 2.9×10^{-6} and 2.9×10^{-5}M GA increases their ability to absorb naptalam by approximately 24 and 26%, respectively. This increase in naptalam uptake is further enhanced by raising the level of GA for pretreatment to 2.9×10^{-4}M. With this concentration 55% more naptalam was absorbed. The peak influence of GA, in this respect, was observed at 6×10^{-4}M, GA-treated plants taking up approximately 58% more naptalam.

The data shown in Figure 1 describes the stimulatory influence of GA on the uptake of naptalam. To obtain this data intact plants were analyzed for naptalam residue. However, this information does not tell us to what extent the different areas of the plant are involved in the uptake and accumulation of the

herbicide. To obtain this information the root system, hypocotyl, lower epicotyl (stem segment between the cotyledonary node and the first true leaves), and the leaf area (aerial portion from and including the first true leaves up) were analyzed separately for naptalam residue.

On a per gram dry weight basis the root systems of plants treated with the lower concentrations of GA used ($2.9x10^{-7}$ to $2.9x10^{-5}$M) accumulated naptalam at a rate comparable to that of control plants (Figure 2). However, as the concentration of GA for pretreatment is raised above $2.9x10^{-5}$M there is a noticeable increase in the amount of naptalam accumulated by the root system. For example, roots from plants treated with $8.6x10^{-4}$GA accumulated almost twice as much naptalam as control plants. (Figure 2).

The accumulation of naptalam by the hypocotyl does not appear to be affected by GA (Figure 2). In addition, no correlation could be established between naptalam accumulation by the lower epicotyl and the concentration of GA used for pretreatment (Figure 2). For example, at $2.9x10^{-7}$M GA the amount of naptalam found in the lower epicotyl was down about 30%, while at $2.9x10^{-5}$M accumultaion of the herbicide was up about 31%. This erratic response was also observed when the higher concentrations of GA were used (Figure 2).

On a per gram dry weight basis the greatest differences

in naptalam accumulation between treated and control plants were found in the leaf area. The leaf areas of all GA-treated plants contained more naptalam than control plants (Figure 2). This effect of GA increased with increase in concentrations of $2.9x10^{-7}$, $2.9x10^{-6}$, and $2.9x^{-5}$M accelerating the uptake and accumulation of naptalam in the leaf area by 31,61, and 73% respectively. However, the most significant increase in herbicide accumulation occurred at $2.9x10^{-4}$M GA. The leaf area of plants pretreated with GA at this concentration contained 212% more naptalam than that found in the control plants (Figure 2). Further increase in concentration of GA ($6x10^{-4}$ to $9.6x10^{-4}$M) for pretreatment only resulted in reducing its stimulatory influence on naptalam accumulation in the leaf area. Nevertheless, the stimulatory effect of these higher concentrations of GA on the uptake of naptalam by the leaf area was considerable (Figure 2).

The synthetic growth hormones, 2,4-D and PCPA, were tested under the same conditions as described above for their influence on the uptake of naptalam by bean plants. The only difference from the GA study was that instead of pretreating the bean plants with growth hormones, the phenoxy compounds were applied simultaneously with the herbicide.

Both 2,4-D and PCPA had stimulatory effects on the uptake of naptalam over a wide concentration range, especially into

the leaf area. As shown in Figure 3, naptalam uptake was accelerated by all concentrations of 2,4-D from $5x10^{-8}$ to $5x10^{-4}$M. Peak stimulatory influence occurred at $3x10^{-5}$M, accelerating uptake by 186%. A sharp drop in the acceleration of naptalam uptake was observed when the concentration of 2,4-D in the root medium was increased from $3x10^{-5}$ to $5x10^{-5}$M. At $5x10^{-5}$M an increase of 76% in uptake was noted. Adding higher levels of 2,4-D, up to 10^{-3}M, only continued to lower the stimulatory influence of the growth hormones on naptalam uptake (Figure 3).

The bean plants were less sensitive to PCPA but, nevertheless, did respond to the growth regulator with an increased uptake of naptalam (Figure 3). Very little influence was observed at the lower concentrations (10^{-8} to $5x10^{-6}$M). However, 10^{-5}M and $5x10^{-5}$M PCPA caused increases of 36% and 71% respectively. Further increases in the level of PCPA to 10^{-3}M served only to decrease the enhancement of naptalam uptake.

The data shown in Figure 3 describe the stimulatory influence of 2,4-D and PCPA on the uptake of naptalam by the entire plant. This type of information does not explain to what extent the different areas of the plant are involved in the uptake of the herbicide. To obtain such information the root system, hypocotyl, lower epicotyl, and leaves were analyzed separately for naptalam residue (Figures 4 and 5).

On a per gram dry weight basis the root system and hypo-

cotyl of plants treated with 2,4-D or PCPA accumulated naptalam at a rate comparable to that of control plants (Figures 4 and 5). The epicotyl, on the other hand, did respond to the growth regulators. Epicotyl tissue of plants treated with 10^{-5}M and $3x10^{-5}$M, 2,4-D contained substantially more naptalam than the same tissue of control plants--233% more with 10^{-5}M and 543% more with $3x10^{-5}$M (Figure 4). This influence of 2,4-D diminished as the concentration of 2,4-D was increased. At the highest concentration (10^{-3}M) uptake of naptalam by the epicotyl tissue was slightly inhibited. Similar to the 2,4-D experiments, naptalam uptake by the epicotyl was accelerated by PCPA, with peak stimulatory influence (160% increase) occurring at 10^{-5}M (Figure 5).

The greatest differences in naptalam uptake were observed when leaf areas of treated plants were compared with those of control plants. Both growth regulators caused highly significant increases in naptalam uptake by the leaf area. Over a range of concentrations from $5x10^{-8}$ to 10^{-3}M 2,4-D large increases in naplalam content were recorded (Figure 4). The peak influence of 2,4-D occurred at $5x10^{-5}$M, the leaves from treated plants containing 575% more naptalam than control leaves. In other words, the leaves of 2,4-D treated plants took up more than five times as much naptalam as those of untreated plants.

Although PCPA was not as effective as 2,4-D, it also caused much greater quantities of naptalam to be taken up by the leaves. Peak stimulation by PCPA occurred at $5x10^{-4}$M, a 487%increase in leaf naptalam content being recorded (Figure 5).

In a related piece of work the patterns of uptake and translocation of tagged naptalam and chloropropham were studied in the cranberry plant. The patterns of C-14 label uptake were similar for both herbicides with the greatest concentration of label found in the roots followed by stems and leaves (Table 1). Chlorpropham was taken up and moved more rapidly in the cranberry plant than did naptalam. As would be expected the uptake of C-14 label increased with length of exposure of the two herbicides (Table 1).

Table 1. Amount of radioactivity extracted from cranberry plants treated with naptalam-C-14 and chlorpropham-C-14

	Radioactivity (dpm/g dry wt)[a]					
	Naptalam			Chloropropham		
	Time after treatment (days)					
Plant Section	1	3	7	1	3	7
Leaves	1,009	1,691	1,627	316	2,061	2,834
Stems	0	2,844	18,728	981	11,081	45,808
Root	14,609	35,775	38,011	335,526	505,329	739,602

a One plant was used for each treatment.

The use of growth regulators in modern agriculture is rapidly growing and the phenoxy compounds and GA are some of the more extensively used compounds of this class. In this study it was shown that bean plants treated with GA, 2,4-D, or PCPA will take up considerably more naptalam than untreated plants. If we assume that other pesticides and plants can be affected in such a manner by these commonly used growth regulators then some problems may be encountered. At the very least a grower planning to use the above mentioned growth regulators should consider what pesticides are present in the soil and what compounds he intends to use immediately before or after he applies the growth regulators.

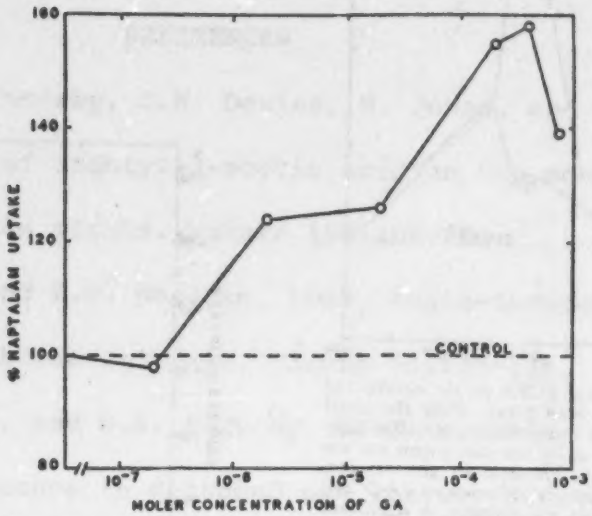

Figure 1. Effect of GA pretreatment on the uptake and accumulation of naptalam by bean plants. The data are given as percents of the control. The stimulatory influence of GA on naptalam uptake and accumulation is significant at the 5% level.

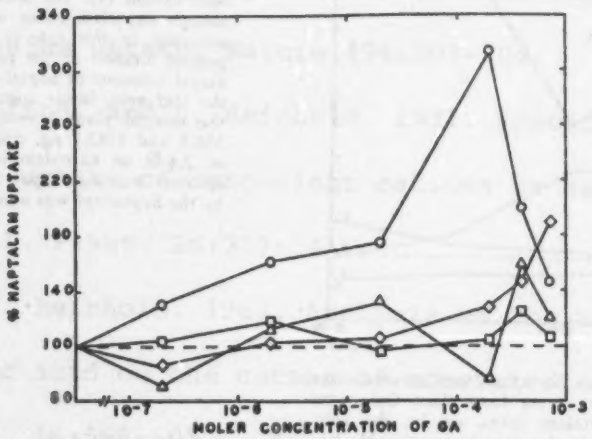

Figure 2. Effect of GA pretreatment on the uptake and accumulation of naptalam by different parts of the bean plant. The data are given as percents of the control. The areas of the plant analyzed for naptalam residue were the root system (◇), hypocotyl (□), lower epicotyl (△), and leaf area (○). The control is the broken line. The stimulatory influence of GA on naptalam uptake and accumulation by the leaf area is significant at the 1% level.

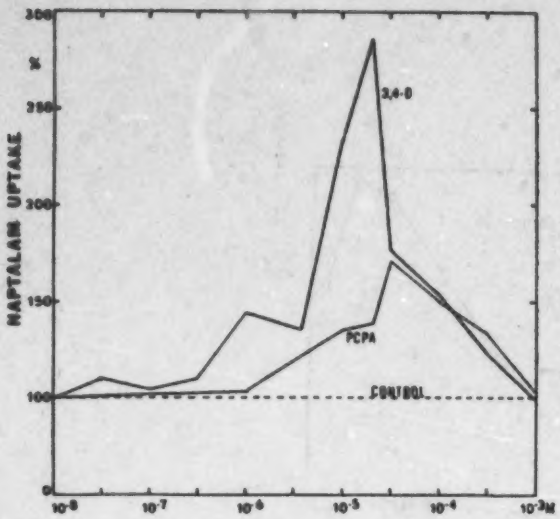

Figure 3. *Influence of 2,4-D and PCPA on the uptake and accumulation of naptalam by bean plants.* Only the aerial portion of the bean plant was analyzed for naptalam since the amount of naptalam taken up by the root system was too great for accurate determination. The data are given as percents of the control. The stimulatory influence of 2,4-D and PCPA on naptalam uptake and accumulation is significant at the 1 % level.

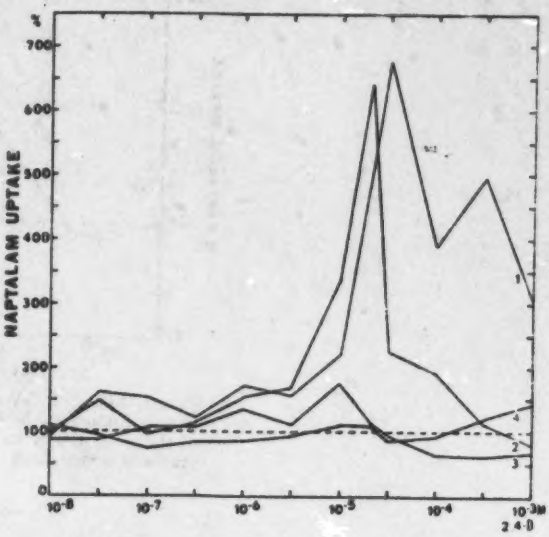

Figure 4. *Influence of 2,4-D on the uptake and accumulation of naptalam by different parts of the bean plant.* The data are given as percents of the control. The parts of the plant analyzed for naptalam residue were the leaf area (aerial portion from and including the first true leaves up) (1), the lower epicotyl (stem segment between the cotyledonary node and the first true leaves) (2), the hypocotyl (3), and the root system (4). The broken line represents the control. Although naptalam taken up by the root system is presented, accuracy in this case is questionable because of the much greater amount of the pesticide taken up by the roots. The actual amounts of naptalam found in one g of dry tissue for the leaf area, lower epicotyl, hypocotyl, and root system of the control plant (average of 36 plants) were 73.8, 252.5, 356.3 and 1382.7 µg, respectively. The stimulatory influence of 2,4-D on naptalam uptake by the leaf area and lower epicotyl was significant at the 1 % level. Increased uptake by the hypocotyl was significant at the 5 % level.

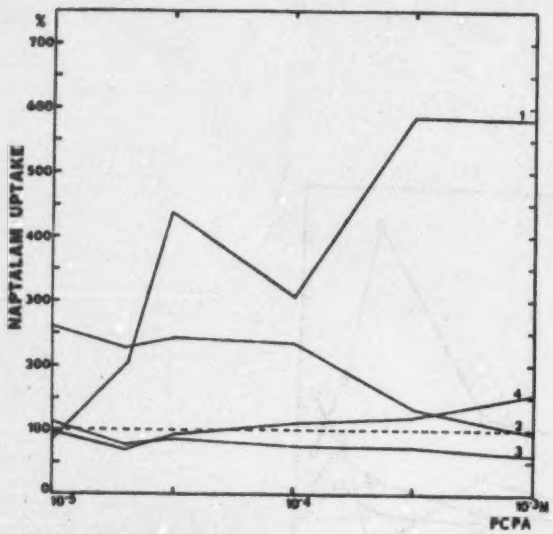

Figure 5. *Influence of PCPA on the uptake and accumulation of naptalam by different parts of the bean plant.* Otherwise see Figure 2. Although naptalam taken up by the root system is presented, accuracy in this case is questionable because of the much greater amount of the pesticide taken up by the roots. The actual amounts of naptalam found in one g of dry tissue for the leaf area, lower epicotyl, hypocotyl, and root system of the control plant (average of 18 plants) were 58.0, 170.9, 343.0, and 1483.8 µg, respectively. The stimulatory influence of PCPA on naptalam uptake by the leaf area and lower epicotyl was significant at the 1 % level. Increased uptake by the hypocotyl was significant at the 5 % level.

SECTION VII

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SECTION VIII

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